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Recurrent *Staphylococcus aureus* Bacteremia

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Sequential blood isolates from eight patients with 10 episodes of recurrent *Staphylococcus aureus* bacteremia were typed by restriction endonuclease analysis of plasmid DNA (REAP DNA fingerprinting) and immunoblotting. There were six early recurrences (within 2 months of stopping antimicrobial therapy) and four late recurrences. All early recurrence isolates were identical to initial isolates. These recurrences were defined as possible relapses. Three of four late recurrence isolates were different from the preceding isolates recovered from four patients. This was considered indicative of new infections. There was complete concordance between REAP DNA fingerprinting and immunoblot typing results. However, four isolates lacked plasmid DNA and could be typed only by immunoblotting. All initial isolates from different patients were different types by immunoblotting and by REAP DNA fingerprinting (except for those lacking plasmid DNA). The bacterial traits detected by these methods appear to be stable in vivo for up to 3 months. Relapsing infections were associated with the presence of intravascular foreign bodies and vancomycin therapy of the preceding episodes.

Multiple new methods to differentiate strains of *Staphylococcus aureus* have been described previously (11). Restriction endonuclease enzyme analysis of plasmid DNA (REAP DNA fingerprinting) and immunoblotting appear to have good discriminatory power and reproducibility (11) and have been successfully applied to the investigation of outbreaks due to methicillin-resistant *S. aureus* (2–4, 7, 12, 13, 16). However, there has been minimal assessment of the in vivo stability of the typing traits detected by these methods (11). Furthermore, plasmids may be gained or lost, and phenotypic assays, such as immunoblotting, may be limited by the capacity of microorganisms to alter the expression of the underlying genes.

This study of sequential blood isolates of *S. aureus* from eight patients with 10 episodes of recurrent *S. aureus* bacteremia provided us with the opportunity to use the two typing methods as tools for clinical assessment. Specifically, we sought to determine whether typing could be used to distinguish relapses due to the original infecting strains from new infections caused by different strains of the same species. Appropriate assessment and management of recurrent bacteremia infections would be aided by the ability to make this distinction.

MATERIALS AND METHODS

Bacterial isolates. All organisms were blood isolates collected at one clinical laboratory serving one medical center (Oregon Health Sciences University) and were identified as *S. aureus* by conventional means (9). Organisms were inoculated into 10% skim milk (Difco Laboratories, Detroit, Mich.) and frozen at −70°C until tested. Bacteremic patients were initially identified by prospective review of blood culture results. Over a 3.5-year period (July 1986 through December 1989), we identified a total of nine patients who had 11 distinct episodes of recurrent *S. aureus* bacteremia. The initial and recurrent episode isolates were available from eight of these nine patients who had 10 recurrent bacteremic episodes. Thus, isolates from 18 bacteremic episodes were typed.

Typing methods. Isolates were assigned code numbers and then blindly processed. Five colonies from subcultures of each isolate were used to inoculate nutrient agar (BBL Microbiology Systems, Cockeysville, Md.) slants which were incubated overnight at 37°C, and five colonies were used to prepare a lawn of cells for REAP DNA fingerprinting. The slants were forwarded to the Long Beach Veterans' Affairs Medical Center for immunoblotting as previously reported (12). The antibody source was, as for previous studies of *S. aureus*, pooled sera from 50 hospitalized patients who were not screened for *S. aureus* infection or colonization. REAP DNA fingerprinting was performed as previously reported (8) at Oregon Health Sciences University. Each isolate was typed by each method and compared with all other isolates in the collection. Recurrences caused by isolates of different types were defined as new infections. Recurrences caused by isolates of identical types were considered possible relapses (although new infection caused by the same strain obviously could not be excluded).

Clinical record review. The inpatient and outpatient records of all patients whose isolates were typed were reviewed. Particular attention was paid to the timing of initial and recurrent episodes, the presence of underlying disease(s), primary and metastatic foci of infection, endocarditis, allergy to antimicrobial agents, antimicrobial resistance of the isolates, the elimination of removable foci of infection, and the drug, dose, and duration of therapy for each bacteremic episode. All of the patients were monitored for at least

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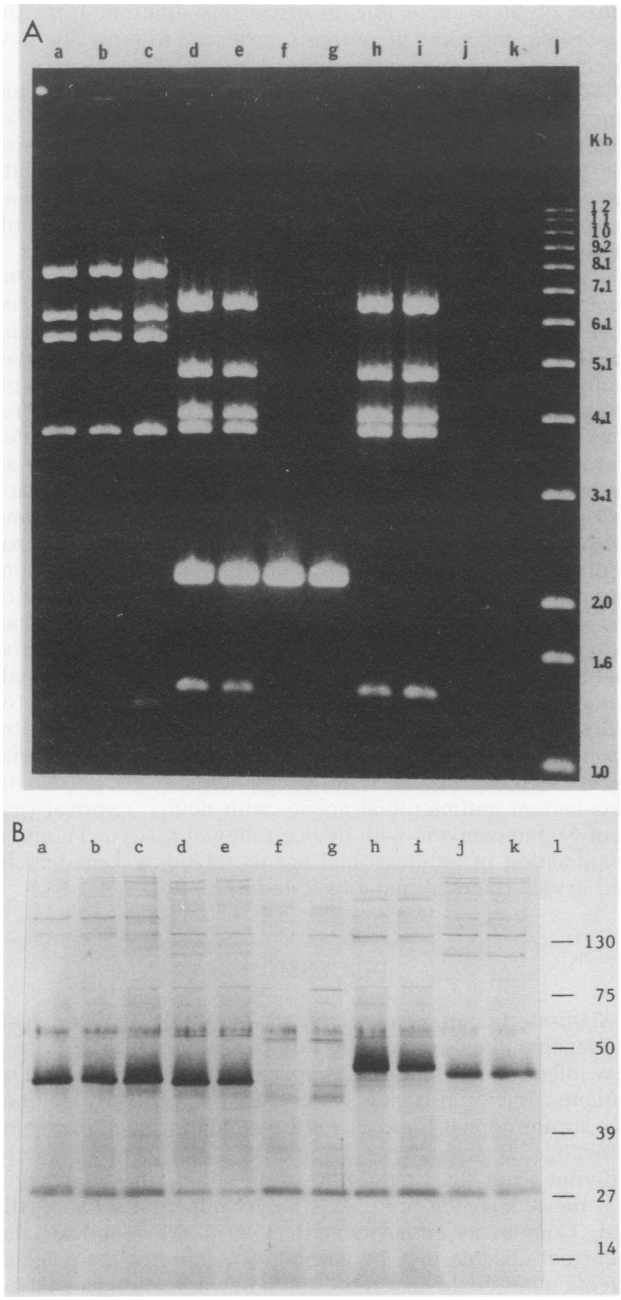

FIG. 1. REAP DNA fingerprints (A) and immunoblots (B) of *S. aureus* isolates from five patients with possible relapses. Electrophoresis followed digestion of preparations with *Hin*dIII. Lanes: a through c, sequential isolates from patient 1; d and e, f and g, h and i, and j and k, sequential isolates from patients 2 through 5, respectively. All isolates are presented in chronologic order. Lane l, 1-kb ladder, with sizes of linear DNA bands (in kilobase pairs) (A) or molecular weight markers (in thousands) (B) on the right.

six months after the last recorded recurrence. None had additional recurrences during this follow-up interval.

RESULTS

Isolate typing. There was a complete concordance of typing results for defining the recurrences in each patient as a possible relapse or a new infection. REAP DNA fingerprints (Fig. 1A) and immunoblots (Fig. 1B) demonstrated identical sequential isolates from five patients who were therefore considered to have possible relapses. One of these patients had three bacteremic episodes with identical isolates (Fig. 1, lanes a to c). The isolates from the fifth patient did not have plasmid DNA demonstrated (Fig. 1A, lanes j and k) but had identical immunoblots (Fig. 1B, lanes j and k). Digests of the plasmid DNA preparations with additional restriction endonuclease enzymes (*Eco*RI and *Xba*I) confirmed the findings of all the *Hin*dIII digests demonstrated in Fig. 1A (data not shown).

REAP DNA fingerprints (Fig. 2A) and immunoblots (Fig. 2B) detected different sequential isolates from three patients who were thus considered as having new infections. Two isolates from these patients' sets lacked linear plasmid DNA (Fig. 2A, lanes a and d) but could be differentiated from the other isolates recovered from these same patients (Fig. 2A, lanes b and c). Differences in immunoblots of the sequential isolates from two patients (Fig. 2B, lanes a and b and lanes c and d) were also readily apparent. The third patient had two recurrences. The first recurrence was considered a possible relapse because isolates were identical (Fig. 2, lanes e and f), whereas the second recurrence was defined as a new infection because of different typing results (Fig. 2, lane g).

There were 8 REAP DNA types and 11 immunoblot types among the isolates examined from the 18 different bacteremic episodes. The strains from different patients that lacked plasmids were differentiated from each other and from other strains by immunoblot typing. Although immunoblotting offered the ability to type all isolates, REAP DNA fingerprinting produced results that were more easily interpreted. Visualized bands were fewer in number and discrete from one another, and molecular size was easy to estimate. These factors were particularly helpful when isolate preparations electrophoresed on different gels were compared.

Clinical record review. None of the patients had recorded or identified allergy to beta-lactam antimicrobial agents. All isolates were susceptible to oxacillin, and all initial and recurrence isolates were susceptible to the antimicrobial agents used for therapy. There was no evidence for recurrence resulting from increased bacterial resistance.

Clinical factors, therapy, classification of the recurrences, and timing of the recurrences are outlined in Table 1. The ages of the eight patients ranged from 19 months to 49 years. Only one patient (patient 5) had an identified primary site of infection that was not related to an intravascular device. This was also the only patient who had an obvious metastatic focus of infection (a septic arthritis); this event occurred during the recurrent bacteremic episode. The other seven patients had intravascular devices in place at the onset of their first episode of *S. aureus* bacteremia. Five had major intravascular lines, one had a prosthetic tricuspid valve, and one had a hemodialysis graft in place. No patient had clinical evidence of endocarditis, although patient 4 was treated for presumed endocarditis because of the presence of a prosthetic heart valve.

Six of the 10 recurrent bacteremias occurred within 33 days of discontinuation of therapy for the preceding episode (or within 68 days of the first positive blood culture from the preceding episode) and were classified as early recurrences. In all of these cases, the sequential isolates were identical types, suggesting possible relapse rather than new infection. Four bacteremias occurred more than 4 months after the preceding episode and were classified as late recurrences.

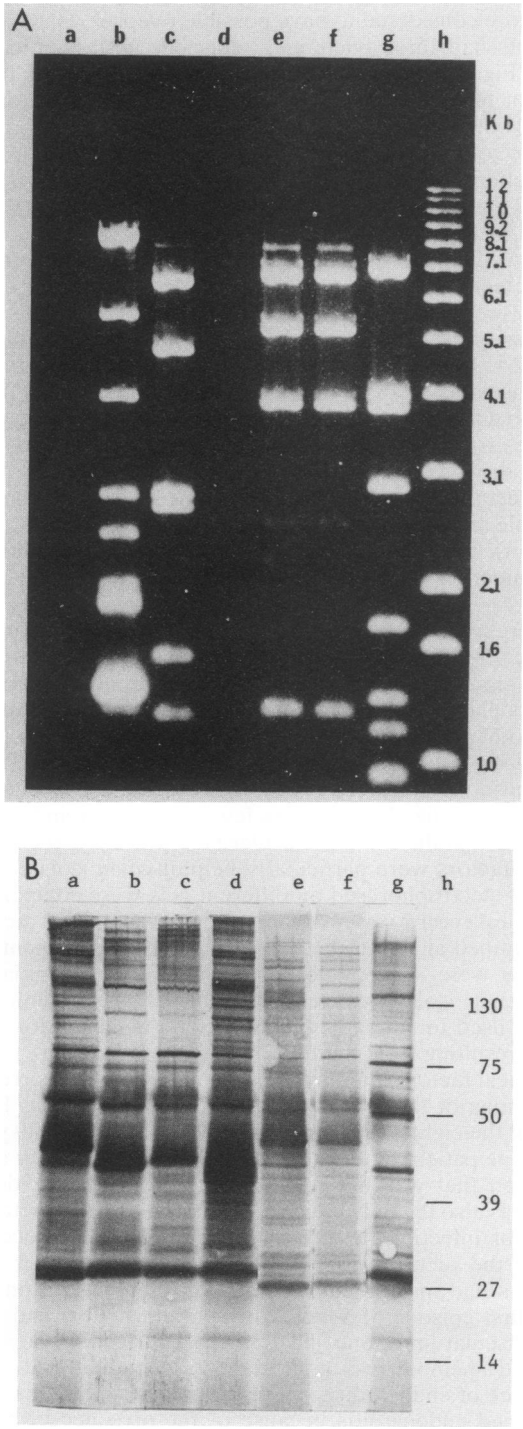

FIG. 2. REAP DNA fingerprints (A) and immunoblots (B) of *S. aureus* isolates from three patients with new infections. Electrophoresis followed *Hin*dIII digestion. Lanes: a and b, c and d, and e through g, sequential isolates from patients 6 through 8, respectively; h, 1-kb ladder (in kilobase pairs) (A) or molecular weight markers (in thousands) (B) indicated on the right.

Three of these were due to organisms different from the preceding strain and were thus considered to represent new infections.

All six patients with early recurrences had received vancomycin (for 14 to 35 days) as the principal agent of treatment for their prior bacteremia. Vancomycin was also the principal therapy that preceded two of the four late recurrences. Except in patient 8 with renal failure, all vancomycin doses were calculated and administered according to a nomogram (10), with a dosing interval of 12 h.

The three late recurrences judged to be new infections occurred in patients who had had removal of the intravascular device that was present at the time of the preceding bacteremia (patients 6 to 8). Two other patients (1 and 3) had recurrences despite device removal, but one of these episodes (patient 1) occurred 7 months later. This likely represented a new infection even though the sequential isolates were identical. All four early recurrences associated with an intravascular device that was present and remained in place following initial therapy were caused by the same strains and were considered possible relapses. Only one of these patients (patient 2) had clinical evidence of device site inflammation or infection at the time of or during the treatment of the initial bacteremic illness. Patient 5 had had a Hickman catheter initially placed after therapy was instituted, blood cultures were negative, and an afebrile state was achieved. An absence of recurrences over 6 or more months of follow-up in the eight patients was finally achieved by regimens comprising primarily beta-lactam antimicrobial agents without device removal (patients 4, 6, 7, and 8), beta-lactam antimicrobial agents with device removal (patient 5), vancomycin with device removal (patient 1), and a combination of antimicrobial agents including vancomycin and device removal (patients 2 and 3).

DISCUSSION

S. aureus bacteremia is common. The ability to distinguish a relapsing illness caused by this common pathogen from a new infection has important implications for management of patients. Bacterial typing systems that can identify distinctive strains appear to be useful for evaluating such recurrent infections. If a recurrence isolate is found to be distinctly different from the original infecting strain, the conclusion that the recurrence represents a new infection seems justified. Conversely, if sequential isolates are identical, the recurrent episode may be caused by relapse of the original infection but could also represent a new infection caused by the same strain. This is especially true for a pathogen like *S. aureus* that may continue to colonize a patient after infection has been resolved.

In our study, all early recurrences (within 33 days from completion of prior therapy and within 68 days from the first positive blood culture of the preceding episode) were caused by the same strain, whereas three of four late recurrences were caused by different strains. This suggests that the early recurrences were true relapses and that the late recurrences were primarily new infections. The fact that one late recurrence occurring 7 months later was caused by a strain identical to the preceding one emphasizes that typing results, like all laboratory tests or tools, must be interpreted judiciously. In this case, new infection caused by the same strain seems the most likely explanation. Another potential problem with the presumption of identity of strains of the same type occurs if the typing tests that are used lack discrimina-

TABLE 1. Clinical factors and therapy for eight patients with recurrent *S. aureus* bacteremia

Patient no.	Age (years)	Bacteremia episode and recurrence no.	Underlying disease(s) and predisposing conditions	Antimicrobic therapy (duration [days])	Status of intravascular device	Relapse or new infection	Interval between episodes
1	12	1, 0	Intestinal pseudo-obstruction, Hickman catheter, parenteral nutrition	Nafcillin (16)	Removed		
		2, 1		Vancomycin (33)	Not removed	Relapse	7.5 months
		3, 2		Vancomycin (134)	Removed	Relapse	51 days
2	49	1, 0	Hodgkin's disease	Vancomycin (14); cephradine (8)	Not removed		
		2, 1		Vancomycin and cefotaxime (14) with gentamicin (3); cephradine (30)	Removed	Relapse	28 days
3	31	1, 0	AIDS, long-arm central IV catheter	Vancomycin (14)	Removed, replaced		
		2, 1		Vancomycin (14); penicillin V and rifampin (90)	Removed	Relapse	33 days
4	29	1, 0	Intravenous drug use, artificial tricuspid valve	Vanomycin (35) and gentamicin (3)	Not removed		
		2, 1		Vancomycin and gentamicin (4); nafcillin (35)	Not removed	Relapse	68 days
5	33	1, 0	Diabetes, renal transplant, infected decubitus	Nafcillin (3); vancomycin (32)	Placed during care		
		2, 1	Plus Hickman catheter	Nafcillin (28); dicloxacillin (21)	Removed	Relapse	60 days
6	1.6	1, 0	Congenital short bowel, Hickman catheter, parenteral nutrition	Vancomycin (14)	Removed		
		2, 1		Nafcillin (21)	Not removed	New	6.5 months
7	9	1, 0	Leukemia, Hickman catheter, chemotherapy, granulocytopenia	Nafcillin and cefotaxime (14); dicloxacillin (14)	Removed and replaced		
		2, 1	Implanted IV catheter, chemotherapy, granulocytopenia	Various (21)	Not removed	New	4.5 months
8	40	1, 0	Hemodialysis, Gortex fistula	Vancomycin and gentamicin (14); vancomycin (14)	Not removed		
		2, 1		Vancomycin (42)	Removed and replaced	Relapse	40 days
		3, 2	Hemodialysis, native fistula	Nafcillin and gentamicin (21); oxacillin (28); rifampin (years)	Not removed	New	9 months

tory power (11). In this study, the two typing methods fully correlated with one another and appeared to have good power for discriminating between unrelated strains.

While an excellent correlation between the two typing systems evaluated was found, each of the systems had a different positive attribute. REAP DNA fingerprinting results were more easily interpreted, but immunoblots provided the ability to type all isolates, including those lacking plasmid DNA. An additional concern is that epidemiologically unrelated isolates may occasionally contain identical plasmids and have identical REAP DNA fingerprints. This result has been demonstrated with isolates from unrelated patients at one institution as well as from different states, countries, and continents (5, 8). Whether such results are also true of immunoblot typing has not been explored. Clearly, REAP DNA fingerprinting and immunoblots evaluate different characteristics of the organisms. In this study, isolates from patients 2 and 4 appear to contain some similar plasmid DNA but seem quite unrelated by immunoblotting, whereas isolates from patients 1 and 2 have more similar immunoblots than REAP DNA fingerprinting results. It will be important to expand such studies with the addition of other typing methods so that the relative merits of different systems can be determined. Systems based on analysis of chromosomal rather than plasmid DNA may be of particular value. Our finding that the early recurrence isolates were identical to the preceding isolates by both typing methods suggests that the typing traits studied are relatively stable in vivo, another consideration in the evaluation of the systems. It is possible that different typing systems may be useful for different purposes. Some of the factors to consider have been reviewed recently (11).

The fact that all of the early recurrences were possible relapses has important therapeutic implications. First, serious consideration must be given to the removal of all indwelling intravascular devices during therapy of patients with bacteremic *S. aureus* infections. Relapses are common among patients with intravascular devices that remain in place, even when the devices do not appear clinically infected (5, 14). Second, vancomycin was used as the primary therapeutic agent for all of our patients who subsequently experienced early possible relapses. The reason for selecting vancomycin was the ease of outpatient administration with less-frequent dosing. This is a practice that is becoming more common (personal observations). Other reports have suggested that vancomycin therapy of *S. aureus* endocarditis may lead to relapse more frequently than therapy with penicillinase-resistant penicillins (1, 15). Our study adds support for the contention that beta-lactam agents are superior to vancomycin therapy of bacteremic *S. aureus* infections. Until large, randomized prospective studies are done to refute this contention, we believe that vancomycin therapy for bacteremic *S. aureus* infections should be limited to patients who are infected with strains resistant to β-lactamase-resistant pencillins or patients who have significant allergies to beta-lactam antimicrobial agents.

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